Comparison of Pulsed and CW Ultraviolet Light Sources to Inactivate Bacterial Spores on Surfaces

Kenneth F. McDonald, *Senior Member, IEEE*, Randy D. Curry, *Senior Member, IEEE*, and Patrick J. Hancock, *Member, IEEE*

***Abstract*—The University of Missouri-Columbia is optimizing photosensitized UV processes for the decontamination of surfaces. The biological inactivation results obtained with high-power pulsed xenon flashlamps are compared to those of a continuous-wave medium-pressure mercury–argon lamp. The inactivation mechanisms associated with pulsed and continuous-wave multispectral light sources, which are used synergistically with a hydrogen peroxide photosensitizer, are discussed. Typically, the process yields more than six logs reduction of *Bacillus subtilis* spores on various surfaces.**

***Index Terms*—Bacillus subtilis, decontamination, disinfection, hydrogen peroxide, microbial inactivation, photosensitizer, surface sterilization, UV light.**

I. Introduction

THE University of Missouri-Columbia (MU), is developing a process that rapidly decontaminates surfaces exposed to microorganisms. A photosensitizer is sprayed on a surface, which is then illuminated with a broadband light source containing strong spectral content below 280 nm (UV-C). Several orders of magnitude reduction in the surviving fraction of microbials is obtained with an incident fluence of a few tens of millijoules per square centimeter [1].

Although several authors have reported that pulsed light kills vegetative cells more efficiently than continuous wave (CW), it is not obvious that spores will respond in the same manner [2]–[4]. Moreover, no reported works compare CW to pulsed lamps in the presence of photosensitizers. The findings of this paper determine that pulsed UV light sources do not produce greater inactivation rates than their continuous wave counterparts when the targets are bacterial endospores inoculated onto surfaces. Neither direct light, nor photosensitized experiments revealed any differences between the microbial inactivation properties of pulsed and CW sources. Inactivation efficacy was determined by bioassay methods as a function of photosensitizer concentration, pulsed and CW UV light sources, and fluence.

II. Methods and Materials

The *Bacillus subtilis* spores, obtained from Dugway Proving Grounds, were prepared by washing five times in sterile deionized water and serially diluted to 5×10^6 cfu/ml. Polystyrene coupons were than inoculated with ten 20-μL drops and air-dried, forming ~3–4 mm spore site diameters. The spore densities, which are in excess of 5×10^{10} cfu/m^2, are exceptionally high compared to those found in typical commercial or military scenarios.

The UV light sources included a 1-kW mercury–argon, medium-pressure CW lamp; and pulsed linear xenon lamps from MU and a private company. Spectra was obtained with a Princeton Instruments Optical Multichannel Analyzer, as shown in Fig. 1. The CW UV source has a broadband light spectrum characterized by strong atomic lines superimposed on a continuum background, while the pulsed sources are characterized by a stronger, pseudoblackbody, continuum with weaker atomic lines.

The light sources were calibrated with a model S310 Scientech Vector laser power meter and an Astral model AC25HD Calorimeter. Cut-on filters, coupled with a subtraction method, were employed to calibrate the UV-C output for each lamp. A family of high-quality Andover cut-on filters was selected for their sharp wavelength cut-on properties. Corrections were made for both the calorimeter response (~85%) and filter transmission characteristics (~90%).

Hydrogen peroxide was the principal photosensitizer used in the presently reported experiments. A surfactant was added to enhance surface wetting, and the solution was sprayed onto target coupons with a Devilbiss Model 163 medical atomizer.

The inoculated surfaces were exposed to a selected flux of either CW or pulsed UV light for varied exposure times, ranging from 4 to 60 s. The exposure to hydrogen peroxide was a constant 75 s, after which, the solution was diluted with sterile water for injection, the spores were recovered, washed three times by centrifugation at $14 \times G$, serially diluted, double cultured on agar and counted 24 and 48 h after exposure. The surviving fraction of the initial concentration is determined by comparison to the cultured colony forming units (CFU) without treatment. The efficacy of photosensitized UV disinfection is compared to that of UV only. Hydrogen peroxide alone was demonstrated to have no effect on *Bacillus subtilis* spores, given the short exposure durations and low concentrations employed in these experiments.

III. Theory of Microbial Inactivation

Reviews of the UV inactivation process are provided by Block [10] and Russell, Hugo, and Ayliffe [11]. The direct UV kill mechanism for vegetative cells is attributed to the photochemical transformation of nucleic acid compounds. The principal inactivation mechanisms include the production

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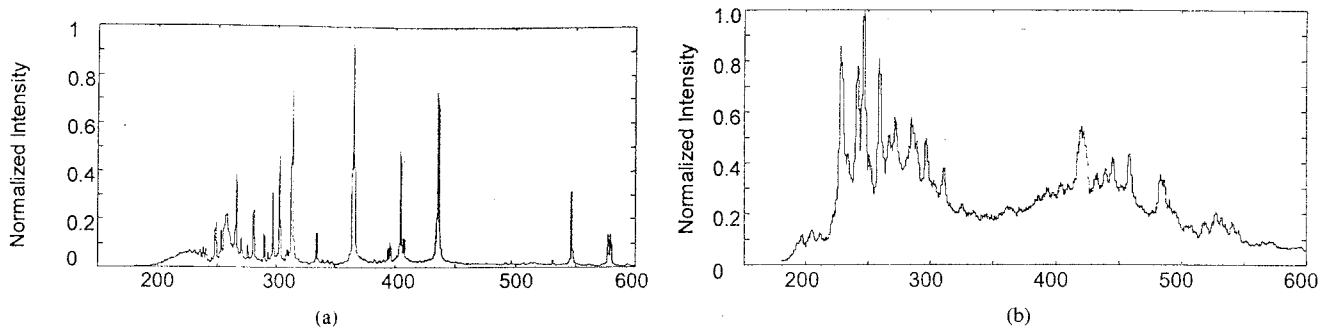

Fig. 1. Spectra from CW and pulsed UV sources. (a) CW spectra from 100 to 600 nm. (b) Pulsed spectra from 100 to 600 nm.

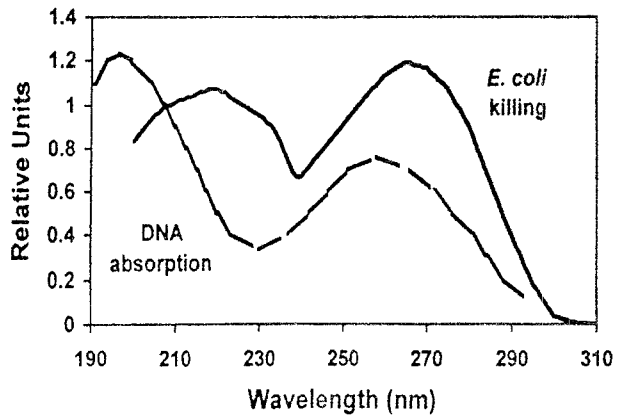

Fig. 2. Biological action spectra and DNA absorbance.

of cyclobutane pyrimidine (predominately thymine-thymine) dimers [5], [6] and DNA strand breaks. The biological damage mechanism is consistent with the absorbance of ultraviolet light by DNA, which peaks in the UV-C band at about 260 nm. The absorbance of DNA and the action spectra for *E-Coli* inactivation are shown in Fig. 2 [7]–[9]. While light in the vacuum UV band (below 200 nm) is absorbed by DNA, it is usually biologically insignificant because of its strong absorbance in the atmosphere.

An indirect biological inactivation mechanism is attributed to the photoproduction of •OH, peroxy, and hydroperoxy radicals in cellular fluids. These highly reactive oxidants attack ligand membranes, proteins, enzymes, and DNA [12]. The production of these radicals and their biocidal effectiveness is especially enhanced when photosensitizers are employed [13]. Thus, photosensitized UV disinfection uses a combination of direct interaction between the UV light and DNA, and an indirect mechanism that involves the photochemical generation of reactive molecules and radicals to inactivate the pathogen.

The UV kill mechanism for spores differs slightly from that of vegetative cells. The DNA is still the principle target, but the damage results not in cyclobutane pyrimidine dimers, but rather in a unique adjunct of a thymine dimer (5-thyminyl-5, 6-dihydrothymine, or TDHT) [14], [15]. Because of the superior repair mechanisms associated with TDHT, spores are much more resistant to the biological effects of ultraviolet light than are vegetative cells [16], [17]. Furthermore, spores are dormant and cannot actively repair UV damage until they undergo outgrowth and germination [18]–[20].

If pulsed power sources yield improved microbial inactivation rates, the enhancement must be attributed to the principal differences between pulsed and CW light sources, which are the peak power (radiant flux) and spectral content. Pulsed xenon flashlamps can easily generate photon fluxes (e.g., >60 W/cm^2 at 30 cm distance) that exceed those produced by medium pressure mercury argon lamps (e.g., 3 nW/cm^2 at 50 cm distance) by four orders of magnitude. Additionally, the spectral envelope of pulsed lamps contains more continuum radiation, and less atomic transition line radiation than medium pressure CW lamps. Finally, the higher operating temperatures of pulsed lamps shift the average spectrum toward shorter wavelengths, i.e., the typical photon energy in the pulse is greater.

Providing both light sources illuminate the target with the same fluence, it is not apparent that pulsed UV sources will impart more damage to spores than CW sources. However, several theories predict a more rapid kill of vegetative cells with pulsed light. The most probable theory postulates that the high photon flux emanating from a pulsed source simply overwhelms the cellular repair mechanisms before repair can be completed. It is also possible that enhanced pulsed UV inactivation rates could be observed with photosensitized experiments because the additional lower wavelength photons increase the production of •OH radicals. This mechanism could enhance the inactivation of spores as well as vegetative cells.

IV. Experimental Results

Given the same dose (fluence) in the UV-C band, experiments with *Bacillus subtilis* spores on polystyrene surfaces were shown to produce identical inactivation results with all three lamps, including the medium pressure CW mercury-argon lamp and both pulsed linear xenon flashlamps.

The CW lamp produces an irradiance of 3.9 mW/cm^2 (in the UV-C) at a distance of 50 cm. Exposure times of 4, 7.5, 15, 30, and 60 s yield fluences ranging from 15.6 to 234 mJ/cm^2. The pulse repetition rate and distance to target for the two flashlamps were adjusted to produce approximately the same average UV-C irradiance as the CW source. For pulse repetition rates of 4.7 and 2.3 Hz, the average UV-C flux for flashlamps #1 (MU) and #2 was ~3.1 mW/cm^2 and ~3.0 mW/cm^2 at target distances of 30 and 35 cm, respectively. Exposure times ranged from 4 to 60 s for all lamps.

The data, shown in Fig. 3, clearly demonstrates that there are no significant differences in the inactivation effectiveness of

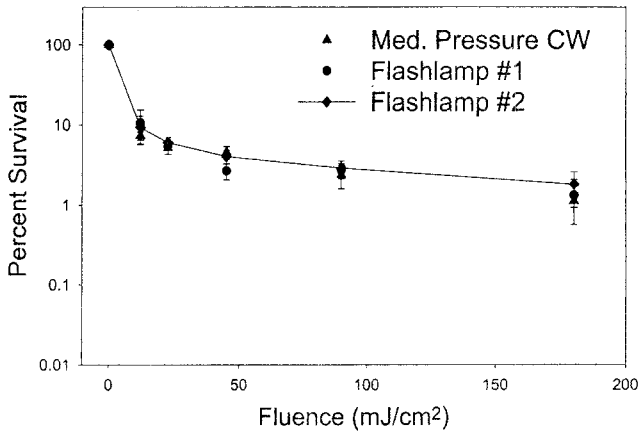

Fig. 3. Inactivation of *Bacillus subtilis* spores on surfaces with CW and pulsed UV light sources.

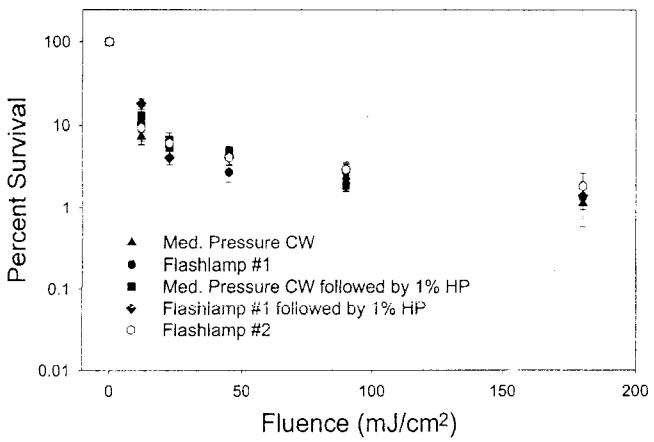

Fig. 4. Inactivation effects of UV only compared to a positive control consisting of UV followed by a 1% hydrogen peroxide photosensitizer solution.

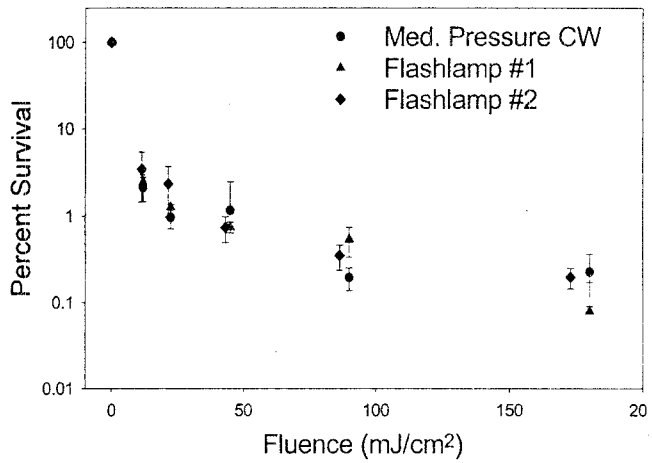

Fig. 5. Synergistically enhanced inactivation of *Bacillus subtilis* spores on polystyrene surfaces by UV light acting in concert with a 1% hydrogen peroxide solution.

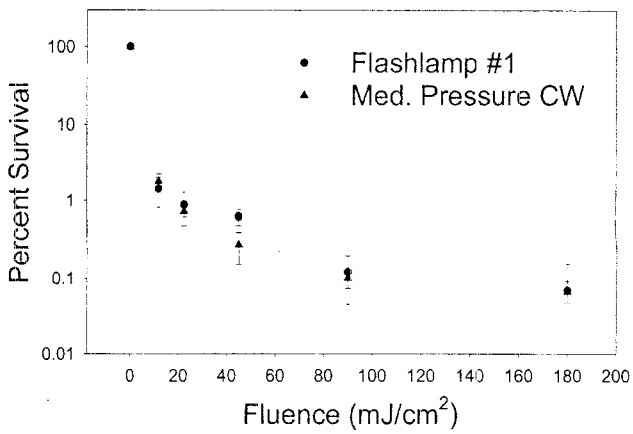

Fig. 6. Synergistically enhanced inactivation of *Bacillus subtilis* spores on polystyrene surfaces by UV light acting in concert with a 2% hydrogen peroxide solution.

spores on polystyrene surfaces with any of the lamps. Multiple data sets are shown for each lamp and the error bars represent the 95% confidence level.

Similarly, photosensitized experiments with a 1% hydrogen peroxide solution (conducted with the same three light sources) produced indistinguishable inactivation results. Sets of positive control experiments compared the inactivation efficacy of the results with light only, to those obtained with light followed by the hydrogen peroxide solution. The data, shown in Fig. 4, clearly demonstrates that the inactivation effectiveness does not change when the 1% hydrogen peroxide solution is applied following exposure to the light sources. It is again observed that no significant differences are manifest between the CW and pulsed sources.

The synergistically enhanced inactivation of *Bacillus subtilis* spores on polystyrene surfaces by ultraviolet light illuminating a thin coating of a hydrogen peroxide based photosensitizer solution is manifest in the data presented in Fig. 5. The enhancement, which is on the order of one log for a 1% H_2O_2 solution, can be observed by comparing the photolytic process data with the positive control results shown in Fig. 4. While it was theoretically possible for the pulsed lights to outperform their CW counterpart because of improved •OH radical production, no such differences were observed.

The effects of increasing the photosensitizer solution from 1% to 2% H_2O_2 are demonstrated by comparing the 1% solution results (shown in Fig. 5) with those obtained for a 2% H_2O_2 solution, as shown in Fig. 6. The synergistic enhancement was seen to increase by approximately 1/2 log of additional kill, but no significant differences were observed in the inactivation efficacy of CW versus pulsed sources.

V. Conclusion

The data obtained in these experiments distinctly demonstrates that the ultraviolet light inactivation of *Bacillus subtilis* spores on polystyrene surfaces is dependent only on fluence (total optical energy per unit area), and is independent of the photon flux over a range of more than four orders of magnitude. Given the same fluence, no differences in the inactivation efficacy were observed when comparing the results obtained with a continuous wave, medium pressure mercury-argon lamp that produces a UV-C photon flux of 3.9 mW/cm^2 on target

to those obtained with pulsed linear xenon flashlamps that produce peak UV-C photon fluxes in excess of 60 W/cm^2 on target. Furthermore, given the same fluence, no microbial inactivation performance difference was observed between the CW lamp and linear pulsed sources for hydrogen peroxide photosensitized experiments over the same four orders of magnitude difference in peak power.

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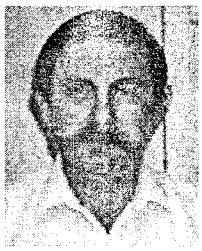

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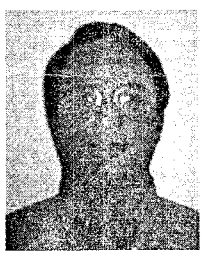

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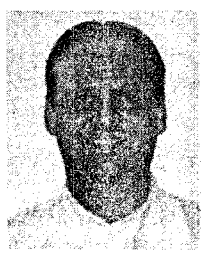
Dr. Curry is a member of the Old Crows Association, American Physical Society, and the American Chemical Society.



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